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# UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.  
48194

Total Pages in this Submission  
73

## TO THE ASSISTANT COMMISSIONER FOR PATENTS

Box Patent Application  
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for invention entitled:

Use and Screening Method For An Aberrant Gene Product-Operating Substance

and invented by:

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: \_\_\_\_\_

Which is a:

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☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: \_\_\_\_\_

Enclosed are:

### Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 47 pages and including the following:
  - a. ☒ Descriptive Title of the Invention
  - b. ☐ Cross References to Related Applications (if applicable)
  - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
  - d. ☐ Reference to Microfiche Appendix (if applicable)
  - e. ☒ Background of the Invention
  - f. ☒ Brief Summary of the Invention
  - g. ☐ Brief Description of the Drawings (if drawings filed)
  - h. ☒ Detailed Description
  - i. ☒ Claim(s) as Classified Below
  - j. ☒ Abstract of the Disclosure

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## Application Elements (Continued)

3. ☐ Drawing(s) (when necessary as prescribed by 35 USC 113)
- a. ☐ Formal Number of Sheets \_\_\_\_\_
- b. ☐ Informal Number of Sheets \_\_\_\_\_
4. ☒ Oath or Declaration
- a. ☒ Newly executed (original or copy) ☐ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)
- c. ☒ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named in the prior application,  
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference (usable if Box 4b is checked)  
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☐ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all must be included)
- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy (identical to computer copy)
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

## Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(B) Statement (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing
- ☐ First Class ☒ Express Mail (Specify Label No.): EL208755144US

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## Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. ☒ Additional Enclosures (please identify below):

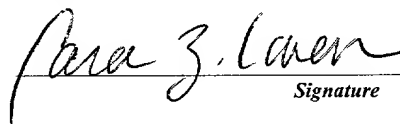
PCT Notification of Record Copy  
Request for Transmittal of Priority Document  
PCT Request  
Associate Power of Attorney

## Fee Calculation and Transmittal

### CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	26	- 20 =	6	x \$18.00	\$108.00
Indep. Claims	8	- 3 =	5	x \$78.00	\$390.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$760.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$1,258.00

- ☒ A check in the amount of \$1,258.00 to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 04-1105 as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of \_\_\_\_\_ as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

  
Signature

Dated: February 25, 1999

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CC:

DESCRIPTION

USE AND SCREENING METHOD FOR AN ABERRANT GENE PRODUCT-  
OPERATING SUBSTANCE

5 Technical Field

10 The present invention relates to a screening method  
for a substance capable of operating an aberrant gene  
product and use thereof. More specifically, the present  
invention relates to a method of creating drugs based on  
the relationship of a gene and the cause of disease," using  
information obtained from analysis of structural genes  
associated with human diseases, e.g., information that  
serves to elucidate the causal relationship between a gene  
polymorphism and a disease, and to a use of a substance  
15 capable of operating an aberrant gene product prepared on  
the basis of said new method of pharmaceutical creation.

Background Art

20 Conventional drug screening technology is based mainly  
on research into the pathologic mechanism of disease in an  
attempt to explore the feasibility of new drug development  
from the viewpoint of action mechanisms. This pathologic  
mechanism represents the outcome, rather than the cause, of  
pathogenesis. For this reason, even a lead compound  
25 obtained from a screening system has often been withdrawn  
from development due to a failure to obtain the desired  
clinical effect, clinical manifestation of unexpected  
toxicity, or another such obstacle. Genome research has  
drawn attention as a new technology since 1990. The vast  
30 genetic information obtained by analytical research into  
genetic information widely involved in biology and medicine  
is expected to provide clues to the elucidation of the  
causal relationship between gene polymorphism and diseases,  
and to enable the identification and creation of drug based  
35 on the cause of pathogenesis.

The process for development of drugs, derived from this genetic information, that act on the cause of pathogenesis, is set forth in the step shown below.

Specifically, this drug development strategy comprises:

- 5 • determining of the sequence and function of a disease-associated gene,
- determining a gene correlated with the cause of pathogenesis and performing functional analysis of genetic information, and
- 10 • selecting candidate drugs on the basis of information on the gene that is the cause of pathogenesis.

Presently, the drug creation approach based on genetic information associated with the genome project is in the initial stage of determining the sequence and function of a disease-associated gene, with efforts being undertaken all over the world to elucidate the relationship between diseases and genetic abnormalities.

Although some disease-associated genes have been reported in Alzheimer's disease and hypertension it will take, considerable time will be taken to completely elucidate the pathology of these diseases because various risk factors remain to be clarified. In obesity, the cause of pathogenesis may soon be elucidated; an anti-obesity drug may soon be developed in the near future. In non-insulin-dependent diabetes mellitus, pathogenetic factors for diabetes may soon be demonstrated at the gene level. In heart diseases, a gene associated with myocardial infarction has already been discovered. These and other achievements suggest it very likely that a drug with a new mechanism of action will be found in near future. In cancer research, efforts to discover genes for etiologic or risk factors have steadily yielded good results. It should be noted, however, that different types of cancer involve high diversity such that more findings must be obtained

before the cause of pathogenesis of each type is completely clarified.

Most patients with Alzheimer's disease (AD) are  
5 solitary cases with unknown hereditary traits. However,  
there are a small number of cases of familial Alzheimer's  
disease (FAD) showing autosomal dominant inheritance. The  
demonstration of the etiologic gene for such FAD would lead  
to the elucidation of the pathology of solitary AD and  
10 subsequent development of a therapeutic drug. Studies  
regarding the FAD gene have recently been conducted  
extensively. As a result, it has been shown to date that  
there is close linkage of the 14th chromosome in many  
families with early onset FAD, and that there is an  
15 abnormality in the  $\beta$  amyloid precursor protein gene,  
located in the 21st chromosome, in a few families.

In contrast, for solitary Alzheimer's disease, which  
accounts for the majority of cases of Alzheimer's disease,  
or familial tardive Alzheimer's disease, the molecular  
20 genetic etiology remained unknown until recent years. In  
recent years, however, studies have been performed based on  
the hypothesis that these diseases may be multiple-factor  
hereditary diseases involved by a number of genetic risk  
factors. In 1993, Corder et al. reported on APO E4, a  
25 polymorphism of the apolipoprotein E gene, as a genetic  
risk factor for Alzheimer's disease (Science, 261, p. 921,  
1993). They analyzed the APO Es in a large number of  
Alzheimer's disease patients and reported out that APO E4  
among those genetic polymorphism for protein is a genetic  
30 risk factor. According to their report, the prevalence of  
APO E4 is significantly higher in the familial delayed  
Alzheimer's disease patient group than in the normal  
healthy subject group. Also, dividing Alzheimer's disease  
patients into 3 groups (those that do not carry APO E4,  
35 those carrying APO E4 as a hetero zygote, those carrying  
APOE4 as a homo zygote) demonstrated that the disease

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develops at younger ages as the prevalence of APO E4 increases.

Also, active studies are ongoing for risk factors other than APO E4, the genetic polymorphism of the VLDL receptor, an apolipoprotein E receptor.

With respect to obesity, the ob protein has recently been identified (Nature, 372, p. 425, 1994) and it has been reported that there is a feedback circuit in which obesity promotes the expression and secretion of the ob protein in fat cells, which in turn stimulates the hypothalamic satiety center to suppress food intake and to increase energy consumption. A study with a hereditary obesity model mouse, known as the ob/ob mouse, is now being conducted to correlate the ob protein (leptin) to human obesity. In addition, the relationship between obesity and the  $\beta 3$  adrenaline receptor is also drawing attention. In other words, obesity can develop due to decreased energy consumption, as well as excessive energy intake.

In humans, as well as in rats and mice, excessive food intake and obesity are thought to potentially stimulate sympathetic nervous system  $\beta 3$  receptor activity to increase energy consumption and cause thermogenesis, especially in brown fat cells etc. This pathway may also be viewed as a feedback mechanism for suppression of the progression of obesity. In cases where such feedback route fails to function due to a congenital anomaly in the  $\beta 3$  receptor, energy consumption is not promoted even by obesity or excessive food intake, resulting in further obesity. In fact, a mutation of the  $\beta 3$  adrenalin receptor by replacement of the 64th Trp residue with an Arg residue has been reported in the Pima Indian tribe which is characterized by high prevalence of obesity and diabetes mellitus [N. Engl. J. Med., 333, p. 343 (1995)]. The same mutation has been identified in people of Caucasian and Finnish descent. This gene mutation, unlike the known gene

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is necessary to completely elucidate the pathology, although genetic factors associated with its pathogenesis have been demonstrated.

Human hypertension is characterized by multiple-factor inheritance, incomplete penetrance and strong influence from environmental factors. Currently, approaches used for genetic analysis using human populations include (1) linkage analysis using the DNA of a family member, in the case of evident familial diseases, (2) the affected sib-pair method, which uses the DNA of an affected sib-pair, and (3) the association study method. Of these methods, linkage analysis is very useful in the identification of causative genes for special forms of hypertension which show a strong genetic background, while the affected sib-pair method and the association study method are used to analyze genetic risk factors in essential hypertension.

Essential hypertension, which presumably accounts for most hypertensive patients, has not undergone extensive linkage analysis, because DNA collection is difficult over 3 generations due partially to slow pathogenesis. In these circumstances, the affected sib-pair method has recently yielded remarkable results.

In 1992, Jeunemaitre et al. identified 15 variations on the angiotensinogen gene, and using the affected sib-pair method for hypertensive sib-pairs in the State of Utah, USA and in Paris, France, demonstrated significant correlation of the M235T (single-base substitution of the 235th codon from Met to Thr) variation in exon 2 and hypertension (Cell, 71, p. 169, 1992).

This correlation was stronger in severe hypertension cases; the mutation was shown to increase blood angiotensinogen concentrations in humans of the TT genotype, as well as the ACD/DD polymorphic genotype.

A report on the correlation of a point mutation (All66C) in the AII1 type receptor gene and hypertension (Hypertension, 24, p. 63, 1994), reports on blood pressure

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the gene level. This does not mean, however, that common cancers are not always solitary or hereditary. Genetic etiologic studies have shown that the relatives of a particular cancer patient have an increased risk of developing of similar tumors. It is therefore reasonable from the viewpoint of multiple-factor genetics to assume that a number of environmental factors and genetic factors serve as prerequisites for oncogenesis.

As stated above, multiple-factor genetic models are applicable to cancers; in fact, cancers are gene-associated diseases. It has been demonstrated that oncogenesis occurs in multiple steps as a result of synergistic accumulation of a number of abnormalities in cancer genes and cancer suppressor genes.

For colorectal cancer, the multiple-step oncogenesis process has been studied extensively. It is assumed that variations in cancer genes such as Ras activate cell proliferation. In contrast an abnormality in the tumor suppressor gene p53 inactivates the functioning of normal genes, resulting in the failure to control cell proliferation.

A group of researchers at Columbia University, USA has recently succeeded in cloning PTI-1, a gene that promotes cell malignancy from prostatic hypertrophy to prostatic cancer. PTI-1 serves as a switch from benign adenomas to malignant tumors.

This factor is a gene that regulates the translation process to affect translation. It is a 46 kd protein homologous to the polypeptide chain elongation factor EF1a (50 kd). PTI-1 is thought to result from point mutation and deletion in EF1. The study by the same group confirmed PTI-1 expression in 15 of their 16 patients with progressive prostatic cancer.

Although efforts to discover genes for the etiologic or risk factors for various diseases have steadily yielded

good results, much information remains to be obtained before the pathogenetic profiles are fully elucidated.

The step of determining the cause of pathogenesis and functional analysis of genetic information subsequent to  
5 determining the sequence and function of a disease-associated gene, is an important task for judging whether the abnormality in the gene is an outcome of disease progression or a pathogenetic cause. As used herein a gene is considered the "cause" of a disease if an aberation  
10 (i.e. mutation) in that gene is correlated with onset of diseases.

However, the information obtained in the previous step (discovery of disease-associated genes) is nothing more than gene sequence information. There are currently almost  
15 no methods to accurately deduce the function of the gene or gene product from this information.

The knock-out transgenic mouse preparation technology may now be the most feasible approach. In the prior art technology, however, unexpected serious damage to the  
20 living body by gene manipulation often results in still-born delivery or neonatal death, which in turn hampers the advance of the study. The knock-out transgenic mouse preparation technology is expected to make major contributions to the analysis of gene function, provided  
25 that there will be further advances.

Although bioinformatics has potential for the clarification of gene function, it is currently in the initial stage of compiling basic information. The speed of gene sequencing has been remarkably increased; the human  
30 cDNA sequence has already been disclosed to a fair degree. During the next several years, advances in the analysis of gene function by a large number of universities and genome venture companies are expected, although their efforts will be accompanied by many difficulties. Such quantitative  
35 increase in basic information is likely to have synergistic effects to dramatically accelerate the analysis of gene

function by bioinformatics after an unknown time point as a powerful tool of functional analysis of genes.

After analysis of the sequence and function of a disease-associated gene, resulting in the identification of the etiologic gene, development of a new pharmaceutical will begin as the third step based on the information obtained from the previous two steps. However, there is no well-established technology for such drug creation.

Summary of Invention

Against this background, there is a need for the establishment of a new technology for identifying and/or creating drugs based on information obtained from analysis of structural genes associated with human diseases that elucidates the causal relationship between a gene polymorphism and a disease. The present invention provides such a new method of pharmaceutical development, and a new use for a substance capable of operating an aberrant gene product on the basis thereof.

After extensive investigation in an attempt to resolve the above problems, the present inventor succeeded in cloning the genes for aberrant gene products (aberrant receptors etc.) from cDNA libraries prepared from human cells or commercially available cDNA libraries derived from human cells, and developed the present invention using cells transformed with those genes.

The present invention relates to a method of creating drugs that enables an approach from the cause of pathogenesis, unlike conventional new drug development approach, which is based on the results of pathogenesis, such as the mechanism of pathogenesis and drug action mechanism. Use of the drug creation technology of the present invention enables a drug creation approach totally differing from the conventional approach. The present invention has a major impact on the development of

therapeutic drugs for diseases associated with the brain/central nervous system, wherein the naturally occurring ligands are of low molecular weights.

More specifically, the present invention provides:

- 5 (1) a pharmaceutical composition comprising a substance capable of causing an aberrant gene product to function in a manner similar to the wild-type gene product, that is, operating, and a pharmaceutically acceptable carrier,
- (2) the pharmaceutical composition according to paragraph
- 10 (1), the substance is an agonist or an antagonist of aberrant gene product,
- (3) the pharmaceutical composition according to paragraph (1), wherein the aberrant gene product is an aberrant receptor, an aberrant channel, an aberrant signal or an
- 15 aberrant enzyme,
- (4) the pharmaceutical composition according to paragraph (1), which contains a substance capable of operating an aberrant operator and which is for prophylaxis and/or treatment of a disease caused by an aberrant receptor,
- 20 (5) the pharmaceutical composition according to paragraph (4), wherein the disease caused by an aberrant receptor is a disease caused by substantial reduction in the activity of the signal transduction system of the cell having the aberrant receptor,
- 25 (6) the pharmaceutical composition according to paragraph (4), wherein the disease caused by an aberrant receptor is a disease caused by substantial absence of the action of a natural ligand on the transfer system of the cell having the aberrant receptor,
- 30 (7) the pharmaceutical composition according to paragraph (4), wherein the disease caused by an aberrant receptor is a disease caused by substantial reduction in the affinity of a natural ligand for the aberrant receptor,
- (8) the pharmaceutical composition according to paragraph
- 35 (6), wherein the signal transduction system is a signal transduction system based on the change in intracellular

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concentration of a responding substance resulting from the binding of a natural ligand and a receptor,

(9) the pharmaceutical composition according to paragraph (6), wherein the responding substance is cAMP, inositol phosphate or calcium ion,

(10) the pharmaceutical composition according to paragraph (4), wherein the substance capable of operating an aberrant receptor is a substance that operates the normal receptor,

(11) the pharmaceutical composition according to paragraph (4), wherein the substance capable of operating an aberrant receptor is a substance that does not operate the normal receptor,

(12) a use for a substance capable of operating an aberrant gene product, which is for treatment of a disease caused by said aberrant gene product,

(13) the use according to paragraph (12), wherein the aberrant gene product is an aberrant receptor,

(14) a method of screening a substance capable of operating an aberrant gene product, which comprises bringing an aberrant gene product into contact with a subject substance and assaying the operation activity of said substance on said product,

(15) the screening method according to paragraph (14), wherein the aberrant gene product is an aberrant receptor,

(16) a method of screening a substance for treatment of a disease caused by an aberrant gene product, which comprises bringing an aberrant gene product into contact with a subject substance and assaying the operation activity of said substance on said product,

(17) a method of screening a drug for substantially operating the signal transduction system of a cell having an aberrant receptor of a mammal suffering from a disease caused by the aberrant receptor, which comprises bringing the aberrant receptor into contact with a subject substance and assaying the operation activity of said substance on said receptor,



(18) the screening method according to paragraph (16), wherein the aberrant receptor is an aberrant receptor prepared by expressing in a cell the gene encoding the aberrant receptor,

- 5 (19) the screening method according to paragraph (16), wherein the gene encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of a gene prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor,

- 10 (20) a method of preparing a drug for treatment of a disease caused by an aberrant gene product, which comprises bringing the aberrant gene product into contact with a subject substance, assaying the operation activity of said substance on said product and preparing a substance judged to substantially operate the signal transduction system of a cell having the aberrant gene product,

- 15 (21) a method of preparing a substance for treatment of a disease caused by an aberrant receptor, which comprises bringing the aberrant receptor into contact with a subject substance, assaying the operation activity of said substance on the aberrant receptor and preparing a substance judged to substantially operate the transfer system of a cell having the aberrant receptor,

- 20 (22) the method according to paragraph (21), wherein the aberrant receptor is an aberrant receptor prepared by expressing in a cell the gene encoding the aberrant receptor,

- 25 (23) the method according to paragraph (22), wherein the gene encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of a gene prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor,

- 30 (23) the method according to paragraph (22), wherein the gene encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of a gene prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor,

- 35 (23) the method according to paragraph (22), wherein the gene encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of a gene prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor,

- (24) a use for an aberrant gene product obtained by expressing in a cell the gene encoding the aberrant gene product, which is for screening for a substance for treatment of a disease caused by said product,
- (25) the use according to paragraph (24), wherein the aberrant gene product is an aberrant receptor,
- (26) the use for an aberrant gene product obtained by expressing in a cell the gene encoding the aberrant gene product, which is for screening for a substance that normally operates said product, and
- (27) the use according to the paragraph (26), wherein the aberrant gene product is an aberrant receptor.

#### Detailed Description of The Invention

The term "aberrant gene product" as used herein includes, but is not limited to aberrant receptors, aberrant channels, aberrant signal and aberrant enzymes. The term "aberrant" above means a mutant in the structural gene corresponding to a gene product, particularly a mutant that has occurred naturally in vivo, which mutant can cause a disease due to the fact that the reactivity of a substance that operates the normal gene product (e.g., the ligand that operates the normal receptor, such as a natural ligand present in vivo) differs from the reactivity of said substance which the normal gene product. Also, it is preferable that the aberrant gene product and the normal gene product are capable of similar of function, i.e., after a substance against the aberrant gene product results in the aberrant gene product acting normally, the aberrant gene product should exhibit a response (e.g., change in intracellular concentration of responding substance in the signal transduction system of cells having the normal receptor) similar to that exhibited by the normal gene product after being operated by an operating substance thereagainst.

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Diseases caused by aberrant gene products include, but are not limited to Alzheimer's disease (e.g., familial Alzheimer's disease, solitary Alzheimer's disease), schizophrenia, depression, hypertension (e.g., essential hypertension), obesity, diabetes mellitus (e.g., non-insulin-dependent diabetes mellitus), heart diseases (e.g., myocardial infarction), cancers (e.g., colorectal cancer, prostatic cancer), rheumatism, allergic diseases (e.g., atopy) and arteriosclerosis, with preference given to Alzheimer's disease, schizophrenia, depression, hypertension, obesity, diabetes mellitus, cancers etc.

The term "aberrant receptor" as used herein is understood to include receptors with a mutation in the structural gene thereof in vivo, resulting in substantially changed affinity for substances such as the natural ligand (e.g., reduction, enhancement etc., preferably reduction), particularly receptors that cause a disease due to the substantial change in the affinity of said natural ligand. The term "substantial change" as used above means a change to the extent that a disease can be caused when the affinity of the natural ligand for the normal and aberrant receptors are compared, and may be any change, whether significant or insignificant, as long as it is capable of causing a disease. Also, it is preferable that the aberrant receptor and the normal receptor be capable of similar function, i.e., after an operating substance against the aberrant receptor results in the aberrant receptor acting normally, the aberrant receptor should exhibit a response (e.g., change in intracellular concentration of responding substance in the signal transduction system of cells having the normal receptor) similar to that product by the normal receptor after being operated by a natural ligand thereagainst. It is preferable that the signal transduction system of cells having the aberrant receptor be identical to that of cells

having the normal receptor. In other words, if the affinity of the natural ligand has been substantially decreased due to a mutation in the receptor gene, even if the cells having the aberrant receptor have the same signal transduction system as that of cells having the normal receptor, the signal transduction system of the cells having the aberrant receptor substantially will fail to function, which can cause a disease. However, according to the present methods, the use of an agonist for the aberrant receptor that binds to the aberrant receptor and which is capable of operating the signal transduction system of the cells having the aberrant receptor would enable the effective prevention or treatment of such a disease.

The disease caused by an aberrant receptor may be any disease, as long as it is caused by a substantial change in the affinity of the natural ligand. Such diseases include diseases caused by substantial reduction in the affinity of the natural ligand for the receptor, diseases caused by substantial failure of the natural ligand to operate the signal transduction system of the cells having the aberrant receptor, diseases caused by substantial reduction in the activity of the transfer system of the cells having the aberrant receptor and diseases caused by aberrant signaling or excessive signaling in the signal transduction system of the cells having the aberrant receptor. More specifically, such diseases include Alzheimer's disease (e.g., familial Alzheimer's disease, solitary Alzheimer's disease), schizophrenia, depression, hypertension (e.g., essential hypertension), obesity, diabetes mellitus (e.g., non-insulin-dependent diabetes mellitus), heart diseases (e.g., myocardial infarction), cancers (e.g., colorectal cancer, prostatic cancer), rheumatism, allergic diseases (e.g., atopy) and arteriosclerosis, with preference given to Alzheimer's disease, schizophrenia, depression, hypertension, obesity, diabetes mellitus, cancers etc.

Substances capable of operating an aberrant receptor include agonist and antagonists to the aberrant receptor and are termed "aberrant receptor agonists" and "aberrant receptor antagonists", respectively. These substances are used as appropriate for the target disease. For example, in diseases caused by substantial reduction in the affinity of the natural ligand for the receptor, diseases caused by substantial failure of the natural ligand to operate the signal transduction system of the cells having the aberrant receptor, diseases caused by substantial reduction in the activity of the transfer system of the cells having the aberrant receptor, and other such diseases, aberrant receptor agonists are preferably used. In diseases caused by abnormal signaling or excessive signaling in the signal transduction system of the cells having the aberrant receptor, and other such diseases, aberrant receptor antagonists are preferably used. Also, the aberrant receptor-operating substance may be a substance that operates the normal receptor or a substance that does not operate the normal receptor, selected depending on the target disease, with preference given to a substance that substantially fails to operate the normal receptor.

Such signal transduction systems include those based on a change in intracellular concentrations of responding substances (e.g., cAMP, inositol phosphate, calcium ion) formed by the binding of a natural ligand and a receptor.

The term "aberrant channel" as used herein is understood to include channels with a mutation in the structural gene thereof, resulting in substantially changed affinity of substances (e.g. blockers, openers) that operate the normal channel, with preference given to channels that cause a disease due to the substantial change in the affinity of said operator. Also, it is preferable that the aberrant channel and the normal channel be capable of similar of function, i.e., after an operating substance



mellitus), heart diseases (e.g., myocardial infarction), cancers (e.g., colorectal cancer, prostatic cancer), rheumatism, allergic diseases (e.g., atopy) and arteriosclerosis, with preference given to Alzheimer's disease, schizophrenia, depression, hypertension, obesity, diabetes mellitus, cancers etc.

The term "aberrant enzyme" as used herein is understood to include enzymes with a mutation in the structural gene thereof, resulting in substantially changed affinity to substances (e.g., activators, inhibitors) that operate the normal enzyme, with preference given to enzymes that cause a disease due to the substantial change in the affinity of said operating substance. Also, it is preferable that the aberrant enzyme and the normal enzyme be capable of similar of function, i.e., after an operating substance against the aberrant enzyme is used on the aberrant enzyme, the aberrant enzyme should exhibit a response (e.g., substrate protein activation) similar to that exhibited by the normal enzyme after being operated by an operating substance thereagainst.

Diseases caused by aberrant enzymes include Alzheimer's disease (e.g., familial Alzheimer's disease, solitary Alzheimer's disease), schizophrenia, depression, hypertension (e.g., essential hypertension), obesity, diabetes mellitus (e.g., non-insulin-dependent diabetes mellitus), heart diseases (e.g., myocardial infarction), cancers (e.g., colorectal cancer, prostatic cancer), rheumatism, allergic diseases (e.g., atopy) and arteriosclerosis, with preference given to Alzheimer's disease, schizophrenia, depression, hypertension, obesity, diabetes mellitus, cancers etc.

Identification of the gene encoding an aberrant gene product (e.g., aberrant receptor) can be achieved by comparing genes prepared from a cell of a mammal (e.g.,

rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, humans, preferably humans) suffering from a disease caused by the aberrant gene product, and genes prepared from a cell of a mammal of the same species that does not have the disease and therefore, does not carry the aberrant gene product.

An aberrant gene identified as a disease-causing gene by analyzing of the sequence and function of the gene (e.g., genes encoding aberrant gene products derived from human cells, preferably genes encoding aberrant receptors derived from human cells, etc.) can, for example, be prepared by the method described below.

First, the RNA encoding an aberrant gene product is prepared from a cell of an animal species from which said gene product is derived (e.g., human cells), preferably cells of animals suffering from a disease caused by the aberrant gene product. Available methods of preparing RNA from such materials include the guanidine thiocyanate method [J.W. Chirgwin et al., Biochemistry, Vol. 18, p. 5,294 (1979)].

After an oligo-dT primer or random oligonucleotide is added to the RNA thus obtained, cDNA can be synthesized in the presence of reverse transcriptase. PCR can be carried out in the presence of a sense primer and antisense primer, both for amplification of the cDNA of an aberrant gene product from a standard cDNA preparation obtained on the basis of a published sequence or analytically identified sequence of said product, PCR can be carried out, as directed in the instruction manual for a commercially available kit (e.g., produced by Cetus/Perkin-Elmer). The amplified cDNA can be separated by a commonly known method, e.g., agarose electrophoresis, and then recovered from the gel. The base sequence of this cDNA can be determined by the dideoxynucleotide synthetic chain termination method [T. Messing et al., Nucl. Acids Res., Vol. 9, p. 309 (1981)].



The plasmid having the cloned cDNA can be used as such, or after being cleaved with an appropriate restriction enzyme and inserted into another vector as desired.

5 Any vector can be used, as long as it enables replication in the host. When the host is a bacterium of the genus *Escherichia* (e.g. *Escherichia coli*), such vectors include *Escherichia coli*-derived plasmids, e.g., pBR322 [F. Bolivar et al., Gene, Vol. 2, p. 95 (1979)], pBR325, pUC12  
10 and pUC13. When the host is a yeast, such vectors include yeast-derived plasmids, e.g., pSH19 [S. Harashima et al., Mol. Cell. Biol., Vol. 4, p. 771, (1984)] and pSH19-1 (European Patent Application Publication EP-A-0235430). When the host is an animal cell, such vectors include, for  
15 example, pSV2-X [R.C. Mulligan and P. Berg, Proc. Natl. Acad. Sci. USA, Vol. 78, p. 2072 (1981)], which results from SV40 ori insertion into pBR322, and pcD-X [H. Okayama and P. Berg, Mol. Cell. Biol., Vol. 3, p. 280 (1983)]. When the host is an insect cell, such vectors include, for  
20 example, the Baculovirus transfer vectors pVL1392 and pVL1393 [manual (MAXBAC<sup>tm</sup> Baculovirus expression system, Manual version 1.4) supplied by the manufacturer (Invitrogen Corporation, CA, USA)].

25 The cloned cDNA may have the translation initiation codon (ATG) at the 5'-terminal thereof and the translation termination codon (TAG, TGA or TAA) at the 3'-terminal thereof. To enhance the expression of said cDNA, it is preferable that the promoter sequence be joined upstream  
30 and operably linked thereto.

Any promoter can be used for the present invention, as long as it is appropriate for the host used to express the desired gene. When the host is *Escherichia coli*, such promoters include the T7 promoter, trp promoter, tac  
35 promoter, lac promoter and  $\lambda$ PL promoter, with preference given to the T7 promoter. When the host is a yeast, such

promoters include the GAPDH promoter, PGK promoter, PHO5 promoter and ADH promoter, with preference given to the GAPDH promoter. When the host is an animal cell, such promoters include the SV40-derived promoter, retrovirus promoter and human cytomegalovirus promoter. When the host is an insect cell, such promoters include the polyhedrin promoter of nuclear polyhedrosis virus.

A promoter can be prepared from the corresponding gene. It can also be chemically synthesized. The expression vector may contain a signal sequence, pre-pro sequence etc., which may be selected from known signals, as long as they function in the host.

Using the thus-constructed DNA-containing recombinant expression plasmid, a transformant is produced.

Hosts include, for example, bacteria of the genus *Escherichia*, yeasts, animal cells and insect cells. Bacteria of the genus *Escherichia* include *Escherichia coli* K12 DH1 [B. Low, Proc. Natl. Acad. Sci. USA, Vol. 60, p. 160 (1968)], C600 [R.K. Appleyard, Genetics, Vol. 39, p. 440 (1954)], MM294 [K. Backman et al., Proc. Natl. Acad. Sci. USA, Vol. 73, p. 4,174 (1976)] and N4830 [M.E. Gottesman et al., J. Mol. Biol., Vol. 140, p. 57 (1980)]. Yeasts include, for example, *Saccharomyces cerevisiae* AH22R<sup>-</sup> [A. Miyanohara et al., Proc. Natl. Acad. Sci. USA, Vol. 80, p. 1 (1983)], NA87-11A, DKD-5D, NA74-3A and NA74-3A<sub>p</sub><sup>-</sup> [Y. Kaisho et al., Yeast, Vol. 5, p. 91 (1989)] and *Saccharomyces pombe* ATCC38399 (h-leul-32) and TH168 (h90 ade6-M210 ural leul) [M. Kishida and C. Shimada, Current Genetics, Vol. 10, p. 443 (1986)]. Animal cells include, for example, simian COS-7 cells, simian Vero cells, Chinese hamster ovarian (CHO) cells, mouse L cells, human FL cells, which are all adhesion cells, and mouse myeloma cells (Sp2/0 cells etc.), mouse YAC-1 cells, mouse MethA cells, mouse P388 cells and mouse EL-4 cells, which are all suspension cells. Insect cells include Sf9 cells.

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Bacteria of the genus *Escherichia* can be transformed in accordance with the method described by T. Maniatis et al. [Molecular Cloning, Cold Spring Harbor Laboratory, p. 249 (1982)], for instance. Yeasts can be transformed in accordance with the method described by A. Hinnen (Proc. Natl. Acad. Sci. USA, Vol. 75, p. 1929 (1978), for instance. Animal cells can be transformed in accordance with the method described by M. Wigler et al. in Cell, Vol. 14, p. 725 (1978), for instance. Insect cells can be transformed in accordance with the manual (MAXBAC<sup>tm</sup> Baculovirus expression system, Manual version 1.4) supplied by the manufacturer (Invitrogen Corporation).

The transformant thus obtained is cultured by a commonly known method.

Media preferably used to cultivate a transformant whose host is a bacterium of the genus *Escherichia* include, for example, the M9 medium containing glucose and casamino acid [J.H. Miller, Experiments in Molecular Genetics, p. 431, Cold Spring Harbor Laboratory (1972)]. To increase promoter efficiency as necessary, a chemical agent such as isopropyl thiogalactoside (IPTG) or indolyl-3-acrylic acid may be added. Cultivation is normally carried out at about 15 to 43°C for about 3 to 24 hours, with aeration and/or stirring as necessary.

Media for cultivating a transformant whose host is a yeast include, for example, Burkholder's minimal medium [K.L. Bostian et al., Proc. Natl. Acad. Sci. USA, Vol. 77, p. 4504 (1980). It is preferable that the medium be adjusted to pH about 5 to 8. Cultivation is normally carried out at about 20 to 35°C for about 24 to 72 hours, with aeration and/or stirring as necessary.

Media for cultivating a transformant whose host is an animal cell include, for example, MEM medium containing about 5 to 20% fetal bovine serum [H. Eagle, Science, Vol. 130, p. 432 (1959)], DMEM medium [R. Dulbecco and G.

Freeman, Virology, Vol. 8, p. 396 (1959)], RPMI-1640 medium [G.E. More et al., Journal of the American Medical Association, Vol. 199, p. 519 (1967)], 199 medium [J.F. Morgan et al., Proceedings of the Society for Experimental Biology and Medicine, Vol. 73, p. 1 (1950)] and ASF104 medium (Ajinomoto). Cultivation is normally carried out at about 30 to 40°C for about 15 to 60 hours, with aeration and/or stirring as necessary.

Media for cultivating a transformant whose host is an insect cell include, for example, TNM-FH medium [W.F. Hink et al., Nature, Vol. 226, p. 466 (1990)]. Cultivation is normally carried out at about 15 to 30°C for about 24 to 72 hours, with aeration and/or stirring as necessary.

According to the present invention, an expression product (aberrant gene product such as an aberrant receptor) can be isolated by appropriate combinations of commonly known methods of separation and purification. Such known methods of separation and purification include those based on solubility differences, such as salting-out and solvent precipitation; those based mainly on molecular weight differences, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis (SDS-PAGE); those based on charge differences, such as ion exchange chromatography; those based on specific affinity, such as affinity chromatography; those based on hydrophobicity differences, such as reverse-phase high performance liquid chromatography; and those based on isoelectric point differences, such as isoelectric focusing.

By expressing an aberrant genetic product by gene engineering technology using *Escherichia coli*, a cultured animal cell, a cultured insect cell etc., the desired product can be produced at high purity and in large amounts.

When the thus-obtained aberrant gene product is in free form, it can be converted to a salt by known methods or a method based thereon. When the aberrant gene product is obtained in salt form, it can be converted to free form or another salt by a commonly known method or a method based thereon.

Also, it is possible to express an aberrant gene product (e.g., aberrant receptor of humans etc.) in a eukaryotic cell using the product's cDNA, and use the aberrant gene product obtained to search for a compound that operates it. Alternatively, by using the gene encoding a specified aberrant gene product as a DNA probe, the mRNA content in the aberrant gene product expressed in vivo can be determined by the Northern blotting method. Moreover, it is possible to prepare an antibody against said aberrant gene product, and quantify said product in vivo by in situ hybridization.

This aberrant gene product can be used for screening for a substance capable of operating an aberrant gene product, screening for drugs for treatment of diseases caused by aberrant gene products (e.g., screening for drugs for operating the transfer system of a cell having an aberrant receptor of a mammal suffering from a disease caused by the aberrant receptor) and for other purposes, and is especially useful for screening for agonists or antagonists for disease-associated aberrant receptors in warm-blooded animals such as humans.

The above screening is hereinafter described more specifically.

The above-described aberrant gene product is useful in searching for an operating substance thereof. In other words, it enables the provision of a screening method for a substance capable of operating an aberrant gene product, wherein said product is brought into contact with a subject

substance to determine the effect of the subject substance on the aberrant gene product.

Useful subject substance include known ligands, synthetic compounds, peptides, proteins etc., as well as  
5 tissue extracts and cell culture supernatants of warm-blooded mammals (e.g., mice, rats, pigs, bovines, sheep, monkeys, humans). For example, such a tissue extract, cell culture supernatant, or the like, may be added to the above-described aberrant gene product, followed by  
10 fractionation with monitoring of operation activity etc., to finally yield a single operating substance.

Specifically, the screening method is exemplified by the method in which substances (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation  
15 products) having operational activity on said product are tested by constructing an expression system for an aberrant gene product (e.g., aberrant receptor) and adding the subject substance to the expression system. In screening for an operating substance for an aberrant receptor, in  
20 particular, it is preferable that an aberrant receptor prepared by expressing in a cell the gene encoding the aberrant receptor be used.

More specifically, the present invention provides a  
25 screening method for a substance capable of operating an aberrant gene product, characterized in that:

- ① the amount of labeled subject substance bound to an aberrant gene product (e.g., aberrant receptor) is determined when the labeled substance is brought into  
30 contact with said product,
- ② the amount of labeled subject substance bound to cells containing an aberrant gene product (e.g., aberrant receptor) or a membrane fraction of said cells is determined when the labeled subject substance is brought  
35 into contact with said cells or the membrane fraction thereof,

- ③ the amount of labeled subject substance bound to an aberrant gene product (e.g., aberrant receptor) is determined when the labeled subject substance is brought into contact with said product expressed on the cell membrane of a transformant containing DNA encoding the product by culturing said transformant,
- ④ a cell-stimulating activity (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) via an aberrant gene product (e.g., aberrant receptor) is used to assay the operation activity of a subject substance when said substance is brought into contact with cells containing said product, and
- ⑤ a cell-stimulating activity (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) via an aberrant gene product (e.g., aberrant receptor) is used to assay the operation activity of a subject substance when said substance is brought into contact with the aberrant gene product as expressed on the cell membrane of a transformant containing DNA encoding the abnormal gene product by culturing said transformant.

Labeled subject substances include substances labeled with [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ], [ $^{135}\text{S}$ ] or other radioisotopes.

Example procedures for screening for a substance capable of operating an aberrant gene product are hereinafter described.

First, a standard aberrant receptor preparation is prepared by suspending cells containing the aberrant receptor or the membrane fraction thereof in an appropriate buffer. Any buffer can be used, as long as it does not interfere with subject substance-aberrant receptor binding. Such buffers include phosphate buffers and Tris-HCl buffers of pH about 4-10 (preferably pH 6-8). For the purpose of reducing non-specific binding, surfactants such as CHAPS, Tween-80 (trade name) (Kao-Atlas), digitonin and deoxycholate, and various proteins such as bovine serum

albumin and gelatin, may be added to the buffer. Also, for the purpose of inhibiting degradation of the receptor and subject substance by protease, protease inhibitors such as PMSF (phenylmethanesulfonyl fluoride), leupeptin, E-64 (produced by Peptide Institute, Inc.) and pepstatin may be added. To 0.01 ml to 10 ml of said receptor solution, a subject substance labeled with a given amount (5,000 cpm to 500,000 cpm) of [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ] or the like is added. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled subject substance in excess is also provided. Reaction is carried out at 0°C to 50°C, preferably 4°C to 37°C for 20 minutes to 24 hours, preferably 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtered through glass fiber filter paper etc. and washed with an appropriate amount of the same buffer, after which the residual radioactivity in the glass fiber filter is measured using a liquid scintillation counter or  $\gamma$ -counter. A subject substance yielding the radioactivity above 0 cpm in count (B - NSB) obtained by subtracting nonspecific binding (NSB) from total binding (B) may be selected as a ligand for the aberrant receptor.

To assess the operation activity of a subject substance on an aberrant receptor, cells containing the aberrant receptor are first cultured on multiwell plates etc. Prior to agonist assay, the medium is replaced with fresh medium or an appropriate buffer that is non-toxic to the cells, followed by incubation in the presence of a subject substance. for a given period of time, after which cells are extracted or the supernatant is recovered, and the resulting product is quantified by a method appropriate thereto. When it is difficult to detect the production of the cell-stimulating activity index substance due to a degradation enzyme contained in the cells, an inhibitor against said degradation enzyme may be added before assay. The operation activity of the subject substance can be



assessed by measuring an activity via an aberrant gene product (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation), or the change in the responding substance concentration in the intracellular signal transduction system (e.g. cAMP, inositol phosphate, calcium ion, or the like), when a subject substance is brought into contact with cells containing said product, or by measuring an activity (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) via an aberrant gene product, or the change in responding substance concentration in the intracellular signal transduction system (e.g. cAMP, inositol phosphate, calcium ion, or the like), when a subject substance is brought into contact with said aberrant receptor expressed on the cell membrane of a transformant containing DNA encoding the aberrant receptor by culturing said transformant.

Also, the present invention provides a method of creating a drug for treatment of a disease caused by an aberrant gene product, wherein the method comprises bringing the aberrant gene product (e.g., aberrant receptor) is into contact with a subject substance, assessing the operation activity of said substance on said product, and preparing a drug judged to substantially operate the aberrant gene product. The present invention also provides a method of preparing a drug for treatment of a disease caused by an aberrant gene product, wherein the method comprises bringing an aberrant receptor into contact with a subject substance assessing the operation activity of said substance on said aberrant receptor, and preparing a drug judged to substantially operate the signal transduction system of a cell containing the aberrant receptor. Thus, a new method of pharmaceutical development or preparation is established based on information obtained from analysis of structural genes associated with human

diseases, which serves to elucidate the causal relationship between a gene mutation and a disease.

The drug thus created can, for example, be used orally in the form of tablets, capsules, elixirs, microcapsules etc., all of which may be sugar coated as necessary, or non-orally in the form of injectable preparations such as aseptic solutions and suspensions in water or other pharmaceutically acceptable liquids. Also, the desired prophylactic/therapeutic agent can be produced by mixing the drug thus obtained with physiologically acceptable carriers, flavoring agents, excipients, vehicles, antiseptics, stabilizers, binders etc. in unit dosage forms for generally accepted manners of pharmaceutical making.

Additives which can be mixed in tablets, capsules etc. include, for example, binders such as gelatin, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint, and cherry. When the unit dosage form is the capsule, the above-mentioned materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical making such as by dissolving or suspending active ingredients, naturally occurring vegetable oils such as sesame oil and coconut oil, etc. in vehicles such as water for injection. Aqueous liquids for injection include physiological saline and isotonic solutions containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol, sodium chloride), and may be used in combination with appropriate dissolution aids such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., polysorbate 80 (trade name), HCO-50) etc. Oily liquids include sesame oil and soybean

oil, and may be used in combination with dissolution aids such as benzyl benzoate and benzyl alcohol.

5 The aqueous liquid may also be formulated with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride), stabilizers (e.g., human serum albumin, polyethylene glycol), preservatives (e.g., benzyl alcohol, phenol), antioxidants etc. The thus-prepared injectable liquid is normally filled in an appropriate ampule.

10 Because the thus-obtained preparation is effective even at low doses, and therefore safe and of low toxicity, it can be administered to warm-blooded mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, humans), for instance. The dose is normally about 0.1 mg

15 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg per day for an adult (weighing 60 kg) in oral administration as a therapeutic agent for hypertension, depending on target disease, symptoms etc. In non-oral administration, it is

20 advantageous to administer the drug in the form of injectable preparation at a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg per administration for an adult (weighing 60 kg), depending on subject of administration, target organ, symptoms, method of administration etc. For

25 other animal species, corresponding doses as converted per 60 kg weight can be administered.

Furthermore, the present invention provides a use of a

30 substance capable of operating an aberrant gene product for treatment of a disease caused by said aberrant gene product (e.g., aberrant receptor), a use of an aberrant gene product obtained by expressing in a cell the gene encoding the aberrant gene product (e.g., aberrant receptor) for

35 screening for a substance for treatment of a disease caused by said product, a use of an aberrant gene product obtained



Pro : Proline (P)  
 Asn : Asparagine (N)  
 Gln : Glutamine (Q)  
 Apr : Ampicillin resistance gene  
 5 Ter : Tetracycline resistance gene

The present invention is hereinafter described in more detail by means of the following examples, which only serve for exemplification and are not to be construed as limitative to the present invention.

10

#### Example 1

##### Cloning of human $\beta 3$ adrenergic receptor cDNA

To amplify human  $\beta 3$  adrenergic receptor cDNA by the PCR method, the following two primers (Nos. 1 and 2) were  
 15 synthesized, with reference to a known nucleotide sequence of the human  $\beta 3$  adrenergic receptor [J.M. Lelias et al., FEBS Lett., Vol. 324, p. 127 (1993)].

Sense primer No. 1:

5'-ATTTGGGAGACCCCTCCTTCCTTCTTTCC-3'

20

(SEQ ID NO:1)

Antisense primer No. 2:

5'-ACAGAGTTGTTGCTTCTTGTCCTTCAGGCC-3'

(SEQ ID NO:2)

Using Takara Ex Taq (Takara Shuzo Co., Ltd.) and the  
 25 included buffer, the following PCR reaction was carried out. Using 0.5  $\mu$ l of a human fat cell-derived cDNA library (CLONTECH Laboratories, Inc.) as the template, 5  $\mu$ l of the attached PCR reaction buffer (10 fold diluted), 4  $\mu$ l of a dNTP mixture, 0.5  $\mu$ l (2.5 U) of TaKaRa Ex Taq, and each of  
 30 2 kinds of primers (combinations of primer No. 1 above and the  $\lambda$ gt11 forward primer (Takara Shuzo Co., Ltd.) and of primer No. 1 above and the  $\lambda$ gt11 reverse primer (Takara Shuzo Co., Ltd.); each 100 pmol) were added to a tube and diluted to 50  $\mu$ l with sterile distilled water. A PCR  
 35 reaction was carried out at 94°C for 2 minutes, 61°C for 1 minute and 72°C for 1 minute in 25 cycles; the 2 tubes were

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mixed; to 5  $\mu$ l of this reaction mixture, 2 primers (Nos. 1 and 2 above; each 100 pmol) were added, followed by a PCR reaction at 94°C for 2 minutes, 55°C for 1 minute and 72°C for 1 minute in 30 cycles. When the PCR product was  
 5 separated by 1% agarose gel electrophoresis, an amplified DNA fragment was confirmed at a position corresponding to the size (1310 bp) expected from the nucleotide sequence of the human  $\beta$ 3 adrenergic receptor. This DNA fragment was recovered from the agarose gel and inserted into the SmaI  
 10 site of the plasmid vector pUC19 (Takara Shuzo Co., Ltd.) to yield pl9-h $\beta$ 3R. The nucleotide sequence of the cDNA was determined by the dideoxynucleotide synthetic chain termination method [T. Messing et al., Nucl. Acids Res., Vol. 9, p. 309 (1981)] and confirmed as identical to the  
 15 published sequence.

#### Example 2

##### Preparation of human $\beta$ 3 adrenergic receptor mutant (Trp64Arg) DNA

20 A synthetic oligonucleotide for mutagenesis was synthesized, and a mutant was generated using the Mutan-Super Express Km (Takara Shuzo Co., Ltd.) as follows:  
 Oligonucleotide for mutagenesis No. 3:

5'-TGGCCATCGCCCGGACTCCGAG-3'

25 (SEQ ID NO:3)

A DNA fragment obtained by digestion of the plasmid pl9-h $\beta$ 3R described in Example 1 with restriction enzymes EcoRI and XbaI was recovered from agarose gel and inserted between the EcoRI and XbaI sites of the plasmid vector  
 30 pKF18k attached to the kit (Takara Shuzo Co., Ltd.) to yield pl8k-h $\beta$ 3R. Using 10 ng of this DNA as the template, in the presence of 5 pmol of the attached selection primer, 5 pmol of No. 5 primer above, 5  $\mu$ l of a reaction buffer (10 fold diluted), 4  $\mu$ l of a dNTP mixture, and 0.5  $\mu$ l (2.5 U)  
 35 of TaKaRa LA Taq, diluted to 50  $\mu$ l with sterile distilled water, a PCR reaction was carried out at 94°C for 1 minute,

55°C for 1 minute and 72°C for 3 minutes in 25 cycles, followed by DNA recovery by ethanol precipitation. This DNA was distilled in 5  $\mu$ l of sterile distilled water; using 2  $\mu$ l of this solution, the DNA was introduced into the attached *Escherichia coli* MV1184 strain to yield transformants capable of growing on kanamycin-containing (50  $\mu$ g/ml) LB plates. Of these transformants, several clones were subjected to nucleotide sequencing at the mutation site; a clone confirmed as having the mutation introduced was designated pl8k-h $\beta$ 3(W64R)R.

### Example 3

Preparation of recombinant DNA for expression of the human  $\beta$ 3 adrenergic receptor gene in animal cells

After the plasmid pl9-h $\beta$ 3R described in Example 1 was digested with EcoRI and XbaI, a human  $\beta$ 3 adrenergic receptor cDNA fragment was recovered from agarose gel. Next, the above-described cDNA fragment was inserted between the EcoRI and XbaI sites of pME18S [R. Sasada et al., Biochem. Biophys. Res. Commun., Vol. 190, p. 1,173 (1993)], a vector for transient expression in animal cells, to yield the expression plasmid ph $\beta$ 3R201. Next, to select a cell line showing stable expression, the neomycin resistance gene (neo), a drug resistance marker, was inserted, as described below. First, a fragment consisting of the SV40 early promoter, the neo gene and the SV40 polyadenylation signal was inserted between the KpnI and SspI sites of the plasmid ph $\beta$ 3R201 to yield the plasmid ph $\beta$ 3R203.

After the plasmid pl8k-h $\beta$ 3(W64R)R described in Example 2 was digested with EcoRI and XbaI, a human  $\beta$ 3 adrenaline receptor variant cDNA fragment was recovered from agarose gel. Following the same procedures as those shown above, the plasmids ph $\beta$ 3(W64R)R201 and ph $\beta$ 3(W64R)R203 were prepared.

## Example 4

Expression of the human  $\beta 3$  adrenergic receptor gene in animal cells

The plasmids described in Example 3 (ph $\beta 3$ R203 and ph $\beta 3$ (W64R)R203) were each introduced into CHO cells (Chinese hamster ovary cells) using the Mammalian Transfection Kit (STRATAGENE, CA, USA) as follows:

To an 80 cm<sup>2</sup> flask, 15 ml of a complete medium [Ham F-12 medium containing 10% (v/v) FCS (fetal calf serum) (LIFE TECHNOLOGIES, INC.)] was added, and  $5 \times 10^5$  CHO cells were seeded. After overnight cultivation in the presence of 5% carbon dioxide at 37°C, this culture medium was replaced with 10 ml of the above medium. After sterile water was added to 30  $\mu$ g of the plasmid described in Example 1 (ph $\beta 3$ R203) to make 450 ml, 50  $\mu$ l of solution #1, then 500  $\mu$ l of solution #2, both included in the kit, were mixed in the 450 ml. After this mixture was kept standing at room temperature for 15 minutes, 1 ml aliquot was added drop by drop onto, and mixed with, the cells, followed by overnight cultivation in the presence of 3% carbon dioxide at 37°C. On the following day, the medium was removed; after the cell surface was washed with F-12 medium (serum-free), 15 ml of a complete medium (10% FCS/F-12) was added, followed by overnight cultivation in the presence of 5% carbon dioxide at 37°C. On the following day, the above cells were seeded at about 200 cells per well to a 12-well plate supplemented with a D-MEM-F-12 medium (Nikken Seibutsu Igaku Kenkyujo) containing 10% FCS, followed by overnight cultivation, after which the cells were further cultured in a medium containing 400  $\mu$ g/ml G418 (LIFE TECHNOLOGIES, INC.) (10% FCS/D-MEM/F-12) until colony formation was noted, with medium replacement every 3 to 4 days. After a large number of colonies were found to form, 1 to several colonies per well were seeded to, and cultured on, a 24-well plate supplemented with a 10% FCS/D-MEM/F-12 medium containing 400  $\mu$ g/ml G418 (2 ml/well), and G418-resistant



cells were harvested (primary cloning). Next, for 2 to 3 wells of the cells harvested in the primary cloning, 10 colonies per well were sown to, and cultured on, a 24-well plate in the presence of G418 at an increased concentration of 800  $\mu\text{g/ml}$ , and G418-resistant cells were harvested. Out of these cells, a line showing high expression of the human  $\beta 3$  adrenergic receptor was selected by the method described in Example 5 below (secondary cloning).

#### Example 5

Determination of cAMP activity in cells showing expression of a human  $\beta 3$  adrenergic receptor

cAMP production activity was determined using the cAMP ELA SYSTEM (Amersham, UK) as follows:

- Each of the culture media of cells showing expression of a human  $\beta 3$  adrenergic receptor obtained on a 24-well plate in Example 4 (CHO/ph $\beta 3$ R203 and CHO/ph $\beta 3$ (W64R)R203) was replaced with a G418-free medium (10% FCS/D-MEM/F-12); 0.5 mM IBMX (3-isobutyl-1-methylxanthine, Wako Pure Chemical Industries) was added. After cultivation in the presence of 5% carbon dioxide at 37°C for 30 minutes, 10-5 isoproterenol [(±)-isoproterenol, Funakoshi] was added, followed by further cultivation for 30 minutes. Next, the cell surface was three times washed with 4°C PBS (phosphate-buffered saline); 300  $\mu\text{l}$  of 0.1 N hydrochloric acid was added, followed by boiling at 95°C for 10 minutes. A 25  $\mu\text{l}$  aliquot was collected from each well and dissolved in 75  $\mu\text{l}$  of the assay buffer attached to the kit. After a 50  $\mu\text{l}$  aliquot of the solution, together with 100  $\mu\text{l}$  of an anti-cAMP rabbit antibody, was added to each well of the kit component 96-well microtiter plate with an anti-rabbit IgG donkey antibody immobilized thereon, the plate was kept standing at 4°C for 1 hour. Next, 100  $\mu\text{l}$  of HRP-labeled cAMP was added; after the plate was kept standing at 4°C for 1 hour, each well was washed 4 times, followed by the addition of 150  $\mu\text{l}$  of TMB (3,3',5,5'-tetramethylbenzidine);

after the plate was kept standing at room temperature for 60 minutes, 100  $\mu$ l of 1.0 N sulfuric acid was added to each well, followed by absorptiometry at 450 nm wavelength. A cell line showing increased cAMP production activity in the presence of isoproterenol, a  $\beta$  adrenergic receptor agonist, was selected.

#### Example 6

Determination of ligand binding activity of a mutant human  $\beta$ 3 adrenergic receptor

A line of Chinese hamster ovary (CHO) cells having the Trp64Arg variant human  $\beta$ 3 adrenergic receptor expressed therein were prepared for use in the search for compounds that specifically bind to the Trp64Arg variant human  $\beta$ 3 adrenaline receptor. After being three times washed with an ice-cooled phosphate buffer, the CHO cells having the Trp64Arg variant human  $\beta$ 3 adrenergic receptor expressed therein were immersed in an ice-cooled hypotonic solution (1 mM Tris-HCl buffer, pH 7.2) for 10 minutes; these after the cells were detached, followed by centrifugation at 4°C and 18,000 rpm for 15 minutes and recovery of the cell membrane fraction, which contained said receptor. After TME buffer (75 mM Tris-HCl buffer, pH 7.4, 12.5 mM magnesium chloride, 1.5 mM EDTA, 4  $\mu$ M desipramine, 5  $\mu$ g/ml leupeptine, 1  $\mu$ g/ml benzamidine, 5  $\mu$ g/ml trypsin inhibitor and 40  $\mu$ g/ml basitlacine) was added, the cell membrane fraction obtained was uniformly suspended using a 25 gauge injection needle to yield a standard receptor preparation. The standard receptor preparation prepared (containing 10 to 30  $\mu$ g, based on protein), [ $^{125}$ I]-iodocyanopindrole (240 pM, DuPont-NEN, USA) and the subject compound were mixed; after adjustment to a final volume of 250  $\mu$ l with TME buffer, the mixture was kept standing at room temperature for 90 minutes. The mixture was aspirated and filtered using a glass fiber filter (GF/B, Packard Instrument Co., Inc., USA) and a cell harvester (Filter Mate Cell

Harvester, Packard Instrument Co., Inc., USA); the radioactivity in the filter was determined using a scintillation counter (TopCount Microplate Scintillation Counter, Packard Instrument Co., Inc., USA). Non-specific binding was quantified in the presence of (S)-(-)-propranolol (Sigma, USA) added to a final concentration of 100  $\mu$ M.

#### Example 7

10 Determination of cAMP-increasing activity in CHO cells expressing a mutant human  $\beta$ 3 adrenergic receptor

A line of Chinese hamster ovary (CHO) cells having the Trp64Arg variant human  $\beta$ 3 adrenergic receptor expressed therein were seeded to a 96-well microtiter plate ( $1 \times 10^4$  cells/well); after 72 hours of cultivation following confluence, the subject compound was added; the plate was kept standing at 37°C for 40 minutes (100  $\mu$ l/well). After the cells were thrice washed with 4°C phosphate buffer, 0.1 N hydrochloric acid was added, followed by boiling at 95°C for 10 minutes. A 25  $\mu$ l aliquot was collected from each well and dissolved in 75  $\mu$ l of the assay buffer attached to the cAMP EIA SYSTEM (Amersham, UK); a 50  $\mu$ l sample was taken from the solution and quantified using the above cAMP EIA SYSTEM, as described below. After the above sample (50  $\mu$ l) and 100  $\mu$ l of an anti-cAMP rabbit antibody were added to a 96-well microtiter plate with an anti-rabbit IgG donkey antibody immobilized thereon, the plate was kept standing at 4°C for 2 hours; after 100  $\mu$ l of horse radish peroxidase (HRP)-labeled cAMP was added, the plate was kept standing at 4°C for 1 hour. After each well was aspirated, the plate was washed 4 times with a washing solution (400  $\mu$ l/well). Next, 150  $\mu$ l of tetramethylbenzidine, an HRP substrate, was added to each well, followed by 60 minutes of incubation during shaking at room temperature. Finally, 100  $\mu$ l of 1.0 N sulfuric acid was added to stop the reaction, after which cAMP was quantified by absorptiometry

at 450 nm wavelength. The results obtained demonstrated that the cAMP concentration in CHO cells increased depending on the concentration of compound added, and that the increase was significant in comparison within the  
5 absence of the compound.

#### Example 8

##### Compound screening

According to compound screening in accordance with the  
10 method of Example 6, a compound showing high affinity for the Trp64Arg variant human  $\beta 3$  adrenergic receptor can be obtained. Testing a compound in accordance with the method of Example 7 reveals that the cAMP concentration in CHO cells showing expression of the Trp64Arg variant human  $\beta 3$ .  
15 adrenergic receptor increase with its concentration.

All references mentioned herein are incorporated by reference. The invention has been described in detail with particular reference to preferred embodiments thereof.  
20 However, it will be appreciated that modifications and improvements with the spirit and teachings of the inventions may be made by those in the art upon considering the present disclosure.

#### 25 SEQUENCE LISTING

SEQ ID NO : 1  
SEQUENCE LENGTH: 30  
SEQUENCE TYPE : Nucleic acid  
STRANDEDNESS : Single  
30 SEQUENCE CLASS : Sense  
SEQUENCE : ACAGAGTTGT TGCTTCTTGT CCTTCAGGCC

SEQ ID NO : 2  
SEQUENCE LENGTH: 30  
35 SEQUENCE TYPE : Nucleic acid  
STRANDEDNESS : Single

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SEQ ID NO      : 3
SEQUENCE LENGTH: 22
SEQUENCE TYPE  : Nucleic acid
STRANDEDNESS   : Single
SEQUENCE TYPE  : Sense
SEQUENCE       : TGGCCATCGC CCGGACTCCG AG

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CLAIMS

1. A pharmaceutical composition comprising a substance capable of causing an aberrant gene product to function in a manner similar to the wild-type gene product and a pharmaceutically acceptable carrier.
2. The pharmaceutical composition according to claim 1, wherein the substance is an agonist or an antagonist of the aberrant gene product.
3. The pharmaceutical composition according to claim 1, wherein the aberrant gene product is an aberrant receptor, an aberrant channel, an aberrant signal or an aberrant enzyme.
4. The pharmaceutical composition according to claim 1, wherein the aberrant gene product is an aberrant receptor and wherein the composition is used for prophylaxis and/or treatment of a disease caused by an aberrant receptor.
5. The pharmaceutical composition according to claim 4, wherein the disease caused by an aberrant receptor comprises a disease caused by substantial reduction in the activity of the signal transduction system of the cell having the aberrant receptor.
6. The pharmaceutical composition according to claim 4, wherein the disease caused by an aberrant receptor comprises a disease caused by the substantial absence of the action of a natural ligand on the signal transduction system of the cell having the aberrant receptor.
7. The pharmaceutical composition according to claim 4, wherein the disease caused by an aberrant receptor comprises a disease caused by substantial reduction in the affinity of a natural ligand for the aberrant receptor.
8. The pharmaceutical composition according to claim 6, wherein the signal transduction system comprises a signal transduction system based on the change in intracellular concentration of a responding substance resulting from the binding of a natural ligand and a receptor.

10. The pharmaceutical composition according to claim 4,  
wherein the substance capable of operating an aberrant  
receptor is a substance that operates the normal receptor.

12. A method of treating a disease caused by an aberrant gene product in an animal, comprising providing to the animal an effective amount of a substance capable of operating the aberrant gene product.

14. A method of screening for a substance capable of  
operating an aberrant gene product comprising bringing an  
20 aberrant gene product into contact with a subject substance  
and assaying the operation activity of said substance on  
said product.

25 16. The screening method for a substance for treatment of a disease caused by an aberrant gene product comprising bringing an aberrant gene product into contact and a subject substance and assaying the operation activity of said substance on said product.

17. A method of screening for a drug for restoring normal function to a signal transduction system of a cell having an aberrant receptor of a mammal suffering from a disease caused by the aberrant receptor, which comprises bringing the aberrant receptor into contact with a subject substance and assaying the activity of said substance on said

receptor and wherein the activity is an activity that restores the normal function of the cell.

18. The screening method according to claim 16, wherein the aberrant receptor is an aberrant receptor prepared by  
5 expressing in a cell the gene encoding the aberrant receptor.

19. The screening method according to claim 16, wherein the gene encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of  
10 a gene prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor.

20. A method of preparing a drug for treatment of a disease  
15 caused by an aberrant gene product, which comprises bringing the aberrant gene product into contact with a subject substance, assaying the activity of said substance on said product and preparing a substance judged to substantially operate the signal transduction system of a  
20 cell having the aberrant gene product wherein said activity is activity that restores wide-type activity of the gene product.

21. A method of preparing a substance for treatment of a disease caused by an aberrant receptor, which comprises  
25 bringing the aberrant receptor into contact with a subject substance, assaying the activity of said substance on the aberrant receptor and preparing a substance judged to substantially operate the signal transduction system of a cell having the aberrant receptor, wherein said activity is  
30 activity that restores wild-type activity to the receptor.

22. The method according to claim 21, wherein the aberrant receptor is an aberrant receptor prepared by expressing in a cell the gene encoding the aberrant receptor.

23. The method according to claim 22, wherein the gene  
35 encoding the aberrant receptor is an aberrant receptor-encoding gene specified by comparative analysis of a gene

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prepared from a cell of a mammal suffering from a disease caused by the aberrant receptor, and a gene prepared from a cell of a mammal of the same species that does not carry the aberrant receptor.

- 5 24. A method of screening for a substance capable of operating an aberrant gene product comprising expressing in a cell the gene encoding the aberrant gene product, separating the aberrant gene product, providing a substance as the aberrant gene product and determining operation  
10 activity of said substance as said gene product.

25. The method according to claim 24, wherein the aberrant gene product is an aberrant receptor.

26. The method according to claim 24 wherein the substance is a substance that normally operates said product.

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ABSTRACT

Provided are uses for a substance capable of operating an aberrant gene product, which is prepared by a new drug creation technology that focuses on the cause of disease using information obtained from analysis of structural genes associated with diseases. Thus, the present invention includes (1) a pharmaceutical composition containing a substance capable of operating an aberrant gene product, (2) a method of using said substance for treatment of a disease caused by said product, (3) a screening method for said substance, and (4) a method of preparing a drug for treatment of a disease caused by said product.

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[illegible]

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S.-Benefit Under 35 U.S.C. §120				
U.S. Applications		Status (Check One)		
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned
PCT Applications Designating the U.S.				
Application No.	Filing Date	U.S. Serial No. Assigned		

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)**  
(35 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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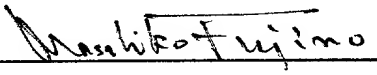
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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201 	Signature of Inventor 202
Date: June 5, 1998	Date:

Signature of Inventor 203	Signature of Inventor 204
Date:	Date:
Signature of Inventor 205	Signature of Inventor 206
Date:	Date:
Signature of Inventor 207	Signature of Inventor 208
Date:	Date:

Docket No. 48194

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Fujino, M.

EXAMINER: Not yet assigned

SERIAL NO. Not yet assigned

GROUP: Not yet assigned

FILED: Herewith

FOR: Use and Screening For an Aberrant Gene Product-Operating Substance

THE HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS  
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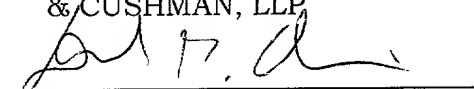
ASSOCIATE POWER OF ATTORNEY (37 C.F.R. 1.34)

As one of the attorneys of record in the above-identified application the undersigned hereby appoints as Associate Attorney for this application the following attorney practicing at Dike, Bronstein, Roberts & Cushman, LLP:

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